Capturing Males of Pestiferous Fruit Flies (Diptera: Tephritidae): Is the Combination of Triple-Lure Wafers and Insecticidal Strips as Effective as Standard Treatments?

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Abstract. The detection of invasive tephritid fruit fly pests relies primarily on traps baited with male-specific lures. Three different male lures are typically used, and accordingly three sets of traps are deployed: those baited with liquid methyl eugenol (ME) or liquid cue lure (CL) for different *Bactrocera* species and those baited with plug-bearing trimedlure (TML) for *Ceratitis* species. The liquid lures contain the insecticide naled, whereas the trimedlure plugs contain no toxicant. Preparing the liquid solutions and servicing three types of traps requires considerable labor, and handling naled (and possibly ME) introduces potential health risks. The purpose of this study was to compare the effectiveness of Jackson traps baited with a solid dispenser (wafer) containing all three male lures plus a separate insecticidal (DDVP; 2, 2-dichlorovinyl dimethyl phosphate) strip with Jackson traps baited with the standard male lure/toxicant combinations. Trapping was conducted during two 12-week periods in a coffee field on Oahu, Hawaii. The effectiveness of the wafer-baited traps varied among different species. Catch of *Bactrocera dorsalis* (Hendel) males was similar between wafer-baited and liquid ME-baited traps for both sampling periods. Conversely, traps baited with the standard TML plug captured significantly more *Ceratitis capitata* (Wiedemann) males than the wafer-baited traps in both sampling periods. The relative effectiveness of the two trap treatments varied between sampling periods for *Bactrocera cucurbitae* (Coquillett) males. Based on these results, the triple-lure wafer plus separate kill strip does not, at present, appear to be a viable substitute for the male lure/toxicant combinations currently in use.

Introduction

Invasive species of tephritid fruit flies (Diptera: Tephritidae) pose a serious economic threat to commercial agriculture worldwide (White and Elson-Harris 1992), and many countries operate trapping programs to detect incipient infestations (Gonzalez and Troncoso 2007, Jessup et al. 2007). Early detection is crucial for effective control, because it increases the probability of limiting the growth and spread of the invasive population and thus may greatly reduce the monetary costs required for eradication or suppression (Lance and Gates 1994). In the US, all southern states maintain surveillance programs, with the most intensive trapping efforts occurring in California, Florida, and Texas (IPRFFSP 2006).

Fruit fly detection traps rely heavily on three male-specific attractants, namely methyl eugenol (ME), cue-lure (CL) and trimedlure (TML). ME and CL attract different species in the genus *Bactrocera*, while TML is attractive to various *Ceratitis* species (Jang and Light 1996). In

particular, among economically important species, ME attracts males of the oriental fruit fly, *B. dorsalis* (Hendel), and closely related species, CL attracts males of the melon fly, *B. cucurbitae* (Coquillett) and the Queensland fruit fly *B. tryoni* (Froggatt); and TML attracts males of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). In US programs, the *Bactrocera* lures ME and CL are applied as liquids (containing the toxicant naled) to cotton wicks, which are then placed in Jackson traps, while TML is presented in polymeric devices (plugs; no insecticide included), which likewise are placed in Jackson traps (Dave Joseph [California], David Dean [Florida], and Hugh Conway [Texas], personal communications).

Current procedures involve two large costs in terms of work efficiency and safety. The use of three different male lures, each placed in a separate trap, results in a large number of required traps, with a concomitant high demand for human and material resources to place and service them. Additionally, the use of liquid *Bactrocera* lures involves considerable handling time for measuring and applying the liquids as well as potential health risks resulting from accidental contact or ingestion of the insecticide. Moreover, data derived from rodents suggest that one of these liquid lures (ME) may be carcinogenic (National Toxicology Program 2000). While implications of these data for human health are uncertain (indeed, ME is a common additive in human food, Burdock 1995), there is increasing concern over limiting worker exposure to ME (Vargas et al. 2009).

Recently, a "wafer" has been developed (Farma Tech International, North Bend, WA) to serve as a solid dispenser for *Bactrocera* male lures, and field tests (Vargas et al. 2009, 2010; Shelly 2010; Leblanc et al. 2011) have demonstrated that traps baited with wafers (containing the lure and the insecticide DDVP, 2, 2-dichlorovinyl dimethyl phosphate) capture as many or more *B. dorsalis* and *B. cucurbitae* males as traps baited with the standard liquid bait plus naled mixture. This basic result has been obtained both for wafers containing either ME or CL (or its natural analog, raspberry ketone [RK]) and wafers containing both attractants. In addition, wafers have been shown to be effective both when the DDVP is embedded in the dispenser itself or presented in a separate strip (Shelly 2013). This latter result is significant, because currently there are no EPA-registered products that contain both an insecticide and ME or CL/RK and that are approved for USDA-APHIS fruit fly surveys (J. Crowe, pers. comm.). Thus, proving the effectiveness of separate lure and insecticide dispensers has important practical implications, as it demonstrates the efficacy of a procedure using solid lure dispensers that is permissible under current regulations.

Initially, the wafers developed contained ME and/or CL/RK exclusively and so focused on *Bactrocera* detection. More recently, however, a wafer has been developed that contains ME, RK, and TML and is thus potentially useful for detecting *Ceratitis* as well as *Bactrocera* and species. Moreover, field tests conducted in Hawaii revealed that traps baited these so-called triple-lure dispensers generally captured as many or more males of *B. dorsalis*, *B. cucurbitae*, and *C. capitata* as traps baited with standard baits (Vargas et al. 2012, Shelly et al. 2012). In both of these studies, the triple-lure wafer was impregnated with DDVP, and at present no data exist regarding the efficacy of traps baited with separate triple-lure and insecticide dispensers relative to standard baits (but see Vargas et al. 2016 for comparisons between triple-lure dispensers with DDVP incorporated or presented in a separate strip).

The objective of this study was to compare captures of *B. dorsalis*, *B. cucurbitae*, and *C. capitata* males in Jackson traps baited with separate triple-lure wafers and DDVP strips with Jackson traps baited with the standard, currently used lure/insecticide treatments.

Materials and Methods

Descriptions of the study site, the traps, and the lures appear in a previous study (Shelly et al. 2012), and here we present a brief summary of the study site and field procedures.

Study site. Field work was conducted in the Dole coffee (*Coffea arabica* L.) field (≈ 65 ha) south of Haleiwa, Oahu, between October–December 2013 and March–May 2015. Average daily minimum and maximum temperatures for the two sampling periods were 22.6°C and 27.8°C and 21.5°C and 26.9°C, respectively (http://www.wunderground.com/ for Wheeler Army Airfield, Wahiawa). All three target species occurred at this location (adjacent host plants served as breeding sites for *B. cucurbitae*, which does not infest coffee). In the coffee field, 30 sampling stations were established in a grid configuration, with 15 stations having standard treatments and 15 stations having triple-lure wafers placed in alternating positions in the grid. Stations were separated by approximately 50 m.

Traps and lures. Jackson traps were used exclusively, and those bearing standard treatments contained the following lure/toxicant combinations: ME- and CL-baited traps each contained one cotton wick to which 6 ml ME (1% naled) or 6 ml CL (5% naled) had been applied, respectively, and TML-baited traps each contained a TML plug (2 g) that contained no toxicant. In a trap, the lure-bearing wick or the TML plug was suspended in a perforated plastic basket in the middle of the trap above the sticky insert. The triple-lure wafers (7.5 by 5.0 cm, 5.0 mm in thickness) contained 5.5 g ME, 2.0 g RK, and 3.5 g TML. Thus, the wafers contained about the same amount of ME as the wicks, but the RK loading in the wafers was markedly lower than the CL loading in the wicks (the specific gravity of both ME and CL is close to 1.0). Conversely, the wafers contained nearly twice as much TML as the plugs. In a trap, the wafer was suspended above the sticky insert by inserting the trap's metal hanger through a pre-made hole in the wafer, and the insecticidal strip (half of a Vaportape II strip, Hercon Environmental, Emigsville, PA; 2.5 by 5.0 cm containing 0.295 g DDVP) was placed in a perforated basket, which was stapled directly to the wafer.

Sampling protocol. Thirty sampling sites, termed stations, were established within the coffee field. Fifteen stations contained standard treatments, and each station included 3 Jackson traps, one each with ME, CL, and TML, respectively. These traps were placed 1.5 - 2.0 m apart on the same or adjacent coffee bushes. While interference among the three lures was possible, any effect was presumed minimal, since prior studies comparing catch in traps baited with triple-lure wafers with traps containing single lures reported qualitatively identical results for the three targeted species regardless of whether the single-lure traps were placed near one another (≈ 2 m; Shelly et al. 2012) or far apart (20 m; Vargas et al. 2012). The other 15 stations contained a triple-lure wafer suspended in a single Jackson trap. Stations were a minimum of 50 m apart. Traps operated for 24 h periods when lures/baits were fresh and after 6, 8, 10, and 12 weeks of weathering. After a sampling interval, the traps were returned to the laboratory, the sticky inserts were removed, and the flies counted. Traps (minus the insert) were suspended

in a shaded area outside the laboratory for weathering until the next sampling period. Trap treatments were alternated between stations over successive sampling intervals.

Ancillary experiments. Based on results described below, two short-term experiments were conducted to investigate the possible negative effect of DDVP strips on the capture of *C. capitata* males. In the first, captures were compared between Jackson traps containing TML plugs alone (standard procedure) and Jackson traps containing TML plugs plus a separate DDVP strip. In the second experiment, captures of male medflies were compared between Jackson traps containing triple-lure wafers with DDVP embedded and Jackson traps containing triple-wafers lacking the insecticide plus a separate DDVP strip. The wafers contained the same amounts of the three male lures and were the same size as described above. In both experiments, the DDVP strip was held in a perforated plastic basket suspended near the lure and was the same size and loading as described above. For each experiment, 15 traps of each treatment were deployed in the coffee field following the protocol described above and operated for 2 d. Two replicates, separated by 7 d and conducted in different areas of the coffee field, were run for each experiment in September-October, 2015 (average daily minimum and maximum temperatures were 20.0°C and 27.8°C, respectively (http://www.wunderground.com/ for Wheeler Army Airfield, Wahiawa).

Data analysis. For both the 2013 and 2015 study periods, captures were analyzed using 2-way ANOVA with week (0 [fresh], 6, 8, 10 or 12 weeks of weathering) and lure type (standard or triple-lure wafer with respective toxicant) as the main factors. Data (x + 1) were $\log_{10}$ transformed values of males captured per trap per day. Separate analyses were conducted for the two years as our focus was on the relative performance of different trap/lure presentations and not temporal variation in fly abundance per se (see Leblanc et al. [2014], who supply this information for Oahu). The normality assumption was met in all cases, but the assumption of equal variances was not. As shown below, this latter finding likely reflected large temporal differences in mean captures, with concomitant differences in the amount of variation among sampling periods. However, in no case was the interaction between week and lure type significant, indicating that, in relative terms, captures in standard lure- vs. wafer-baited traps were similar over time. Thus, we considered ANOVA sufficiently robust to assess potential differences in captures between traps baited with standard lures vs. triple-lure wafers. Pair wise comparisons in the ancillary experiments were made using a t-test with $\log_{10}$ transformed data (X + 1) as with this transformation the assumptions of the test were met. Means $\pm$ 1 SE are given.

Results

October–December 2013. For both *B. dorsalis* and *B. cucurbitae*, sampling week had a significant effect on male captures, but lure type did not (Table 1, Fig. 1). However, both week and lure type had significant effects on captures of *C. capitata* males (Table 1; Fig. 1). Greater numbers of C. *capitata* males were captured in traps baited with TML plugs than those baited with the triple-lure wafers on all sampling weeks. Temporal trends in captures of C. *capitata* were similar between traps baited with TML plugs or triple-lure wafers, with captures being high initially, declining in weeks 6 and 8, and then increasing in the final sampling weeks.

March–May 2015. For *B. dorsalis*, sampling week had a significant effect on male captures, but lure type did not (Table

Table 1. Results of 2-way ANOVA for trap captures of fruit fly males in the 2013 and 2015 study periods. F values are given; degrees of freedom for week were 4, 149; for lure type were 1, 149; and for week x lure type interaction were 4, 149.

Year	Species	Week	Lure type	Interaction
2013	*C. capitata*	13.5 (P < 0.001)	6.9 (P = 0.01)	0.7 (P = 0.60)
	B. dorsalis	281.6 (P < 0.001)	2.2 (P = 0.14)	2.3 (P = 0.06)
	B. cucurbitae	8.4 (P < 0.001)	2.2 (P = 0.15)	0.6 (P = 0.68)
2015	*C. capitata*	15.9 (P < 0.001)	84.7 (P < 0.001)	2.2 (P = 0.07)
	B. dorsalis	74.5 (P < 0.001)	0.1 (P = 0.71)	0.9 (P = 0.50)
	B. cucurbitae	63.1 (P < 0.001)	224.1 (P < 0.001)	0.1 (P = 0.99)

1, Fig. 2). However, both week and lure type had significant effects on captures of *B. cucurbitae* and *C. capitata* males (Table 1; Fig. 2). For *B. cucurbitae*, traps containing liquid CL captured 8-11 males per trap per day over the 2015 study period (excepting week 0), whereas traps with the triple-lure wafer caught only 1–3 males per trap per day. For *C. capitata*, traps with TML plugs captured 20–30 males per trap per day over the entire sampling interval, whereas traps with the triple-lure wafer caught only 6–19 males per trap per day.

Ancillary tests. There was no significant difference in the number of male medflies captured in traps baited with TML plugs alone or TML plugs plus a DDVP strip (males per trap per day: Replicate 1: 75.0 ± 7.3 vs. 73.2 ± 7.8, respectively, t = 0.2, P = 0.83; Replicate 2: 17.3 ± 2.5 vs. 17.0 ± 1.5, respectively, t = 0.3, P = 0.76). Likewise, similar numbers of male medflies were captured in traps baited with triple-lure wafers containing DDVP or with triple-lure wafers lacking DDVP but with a separate, nearby insecticidal strip (males per trap per day: Replicate 1: 40.6 ± 4.0 vs. 37.0 ± 3.9, respectively, t = 0.8, P = 0.45; Replicate 2: 16.1 ± 1.5 vs. 14.3 ± 1.4, respectively, t = 0.9, P = 0.35). Although attention was focused on *C. capitata*, it should be noted that for both replicates male captures of both *B. dorsalis* and *B. cucurbitae* were not significantly different between triple-lure wafers with DDVP or triple-lure wafers lacking DDVP but with a nearby DDVP strip.

Discussion

Previous studies (Vargas et al. 2012, Shelly et al. 2012) found that Jackson traps containing wafers containing TML, ME, RK, and the insecticide DDVP generally captured similar or greater numbers of *C. capitata* and *Bactrocera* spp. males as Jackson traps baited with standard treatments for each of the three lures. In contrast, the present findings show that the

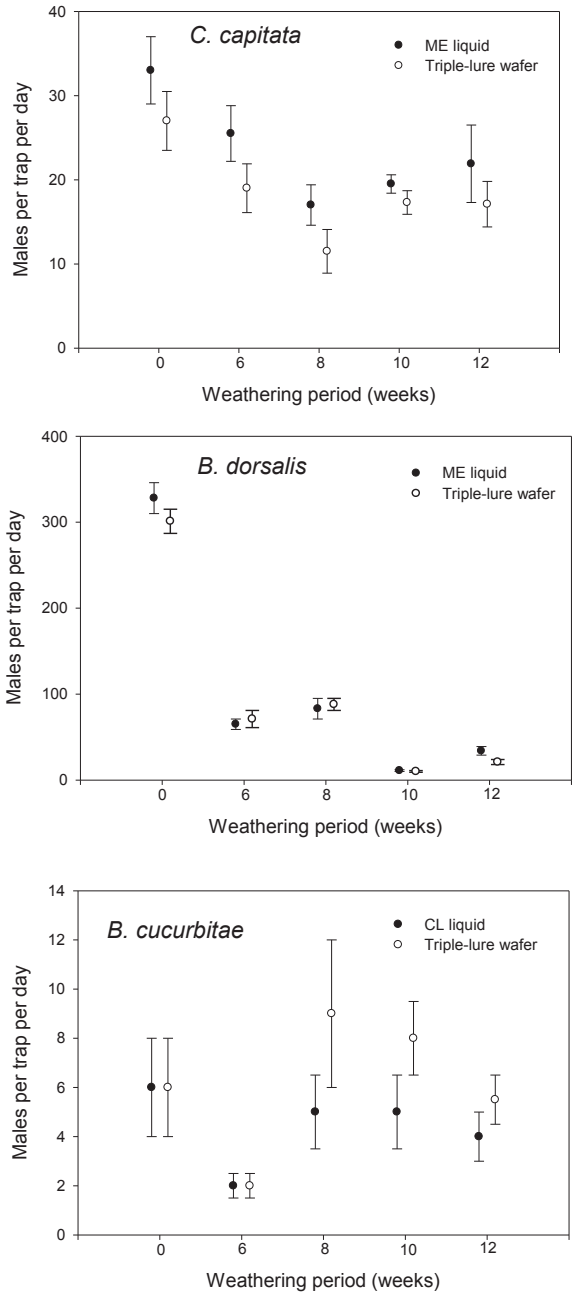

Figure 1. Captures of *Ceratitis capitata*, *Bactrocera dorsalis*, and *Bactrocera cucurbitae* males in Jackson traps baited with standard lures or triple-lure wafers over a 12-week interval in October-December, 2013, in a coffee field near Haleiwa, Oahu. Symbols represent mean values of 15 traps; error bars represent 1 SE.

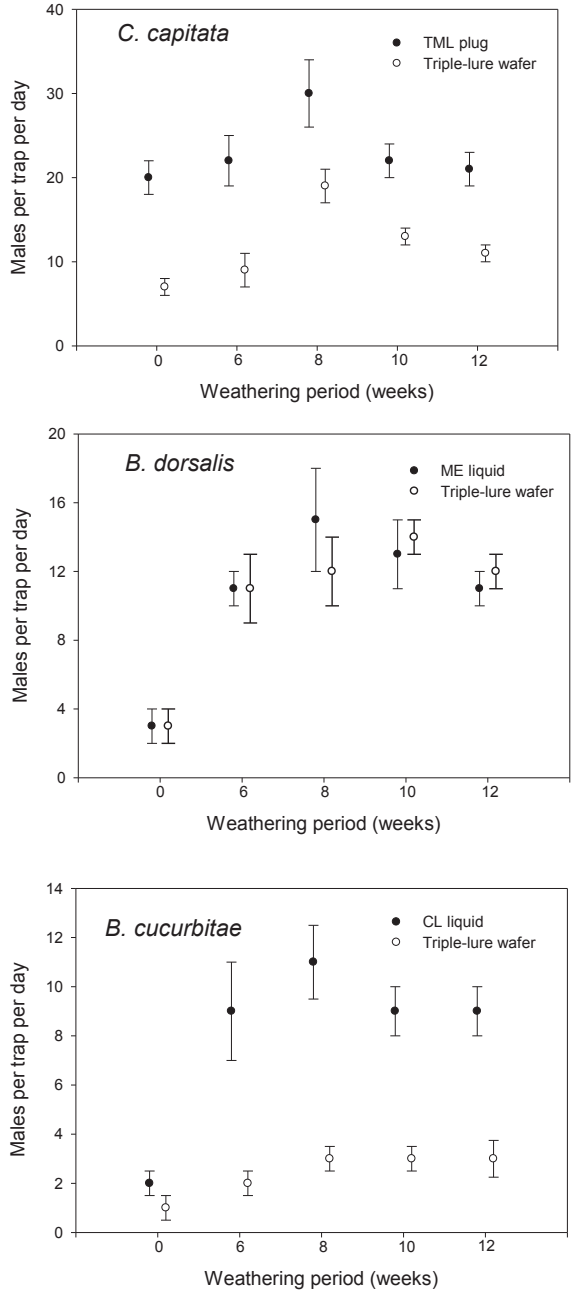

Figure 2. Captures of *Ceratitis capitata*, *Bactrocera dorsalis*, and *Bactrocera cucurbitae* males in Jackson traps baited with standard lures or triple-lure wafers over a 12-week interval in March-May, 2015, in a coffee field near Haleiwa, Oahu. Symbols represent mean values of 15 traps; error bars represent 1 SE.

Table 2. Loadings of the male lures trimedlure (TML), methyl eugenol (ME), and raspberry ketone (RK) and the killing agent DDVP in triple-lure wafers used in three different studies. All values are g/wafer.

Study	TML	ME	RK	DDVP
Vargas et al. (2012)	2.4	3.5	2.4	0.6
Shelly et al. (2012)	2.3	4.4	2.3	0.5
Present study	3.5	5.5	2.0	0.0*

*Separate strip with 0.295 g DDVP.

triple-lure wafers plus a separate DDVP did not perform as effectively as the standard treatments. While captures of *B. dorsalis* males were comparable between standard and wafer-baited traps, traps baited with the triple-lure wafers captured significantly fewer *C. capitata* males than standard TML plugs in both study periods and significantly fewer *B. cucurbitae* males in one of the study periods (2015). Comparisons among the different studies are potentially confounded, because they used wafers of varying composition (Table 2). However, it is not immediately obvious that this variation accounted for the differing results.

Perhaps the most noticeable difference among the studies is the amount of DDVP present in traps baited with triple-lure wafers. In instances where the triple-lure wafer also contained DDVP, the amounts of the toxicant present were 0.6 g (Vargas et al. 2012) and 0.5 g (Shelly et al. 2012), respectively. In contrast, the separate kill strip used in the present study contained 0.295 g DDVP. While this lower DDVP dose could have accounted for the poorer performance of the triple-lure wafers in the present study, several lines of evidence suggest it was not a critical factor. First, the relatively low amount of DDVP contained in the strips used in the present study did not result in lower capture of *B. dorsalis* males in either 2013 or 2015 study periods. If the low amount of DDVP were a factor, then its effect would presumably be evident for all three fruit fly species captured (assuming similar susceptibility to DDVP among them), but this was obviously not the case. Second, an experiment (Shelly et al. 2015) explicitly testing the effect of field weathering on the potency of DDVP strips reported that, even after 12 weeks of weathering in the summer heat of Florida and Arizona, small DDVP strips containing only 0.09 g of DDVP were as effective in trapping *Bactrocera* spp. as freshly deployed (non-weathered) DDVP strips of the same type or naled mixed with liquid lures. These results suggest that the lower DDVP amount in the wafer-baited traps was not responsible for the relatively poor performance of these traps in capturing *B. cucurbitae* in 2015. Finally, the finding that, in both 2013 and 2015 study periods, traps baited with TML plugs alone without any toxicant (i.e., the standard treatment) captured significantly greater numbers of *C. capitata* males than traps with wafers and a DDVP strip suggests that the DDVP strip possibly acted as a repellent. Katsoyannos et al. (1999), for example, reported that placement of a DDVP-bearing plug in wet, food-baited McPhail traps resulted in lower captures of medflies than similar traps lacking these plugs. However, the relevance of this finding to the present study is uncertain, because the DDVP concentration in the plugs was not specified and both a different trap type and lure were used (see also Jang 2011). More directly, of course, our ancil-

lary experiments revealed no difference in the number of male medflies captured in Jackson traps containing (i) a TML plug alone or with a DDVP strip present or (ii) a triple-lure wafer containing DDVP or a triple-lure wafer containing the attractants only with DDVP presented in a separate strip. Based on these findings, it appears unlikely that DDVP, when presented separately, acted as a repellant to male medflies in the present study (but see Vargas et al. [2016] for discussion of possible DDVP repellency to *C. capitata*).

Regarding the lures themselves, it does not appear that dose differences accounted for the poor performance of the triple-lure wafers in the present study (Table 2). The wafers used in the present study contained a similar amount of RK and a greater amount of TML as those used in previous studies (Table 2), indicating that the low captures of *B. cucurbitae* (2015) and *C. capitata* (2013, 2015) did not reflect inadequate lure doses. Conversely, the similarity in trap catch of *B. dorsalis* males between wafer- and liquid-baited traps noted herein did not depend on the comparatively high ME loading: the ME dose in wafers used by Shelly et al. (2012) was 80% that in wafers used in the present study, yet captures of *B. dorsalis* males were similar between wafer- and liquid-baited traps in that earlier study. As noted above, the wafers tested here contained 75% more TML than the plugs (3.5 g vs. 2.0 g), yet it seems unlikely that the relatively low catch of male *C. capitata* reported here reflected a repellant effect of a high TML dose in the wafers. In an independent test (Shelly, unpublished data), similar numbers of male medflies were captured in Jackson traps baited with 5 ml (≈ 5 g) of liquid TML and Jackson traps baited with 2 g TML plugs.

In conclusion, with respect to *C. capitata* in particular, we have no adequate explanation for the poor performance of the triple-lure wafer plus separate kill strip compared with the standard treatments. Neither differences in the amounts of lures and insecticide deployed nor the method of DDVP presentation (embedded in wafers or presented separately) appear to account for this finding. Moreover, the fact that the wafer plus separate kill strip resulted in significantly fewer captures of *C. capitata* than the standard treatment in two distinct sampling periods makes it unlikely that some unique, or unusual, set of environmental factors was responsible for this result. While problematic for detection of *C. capitata*, this study plus several others (Vargas et al. 2009, Jang 2011, Shelly 2013, Shelly et al. 2015) indicate that wafers with male lures plus a separate DDVP strip are effective for *Bactrocera* species, which would eliminate the handling of liquid lures and toxicant in trapping efforts for these pests.

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